Synopsis

Ph.D. in Biochemistry

Neuroprotective effect of synthesized zinc oxide Nanoparticle-capped catechin

Submitted by

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INTRODUCTION

Neurodegenerative diseases have become a menace in the 21st century and they are more prevalent in the old age population. Occurrence of neurodegenerative diseases is increasing all over the word due to increased proportion of old age population. Development of neurodegenerative diseases in old age people is systematic and finally leads to death (Yadav, 2021). Neurodegenerative diseases are estimated to be the second most common cause of death among elderly by the 2040's (Bachari *et al.,* 2024). A number of lifestyles such as physical activity, diet, intellectual engagement and social interactions are associated with the risk of age-associated neurodegenerative disorders (Kim *et al.,* 2019). Current therapies for neurodegenerative diseases provide symptomatic relief of particularly in earlier stages of the disease. At the late stages of neurodegenerative diseases, the drugs fail to cross the blood brain barrier (BBB), which acts as a formidable porter towards the access of exogenous substances into the brain and makes the treatment unsuccessful (Poovaiah *et al.,* 2018). Acetyl cholinesterase inhibitors are first-line medications for the treatment of neurodegenerative diseases and are associated with mild improvements in cognitive function, behaviour and activities of daily living (Walczak-Nowicka and Herbet 2021).

One of the biomarkers of neurodegenerative diseases is decreased acetylcholine (ACh) concentration and function, which is associated with memory and learning (Ayaz *et al*., 2020). There are different antioxidants in the brain. Actually, antioxidant activity of the brain is lower than in other tissues. So, neural cells are more susceptible than other tissues to oxidative damage (Tewari *et al.,* 2014 and Mohebbati *et al.* 2017). Normally, small traces of reactive oxygen species may not be toxic, but excessive amounts may lead to oxidative stress, which is the primary factor in neurodegenerative diseases (Junsathian *et al.,* 2018). Antioxidants can reduce reactive oxygen species production, tissue damage and improve cell survival. Chemical compounds in plants known as phytochemical compounds can contain phenolic and flavonoid groups, which have potential health benefiting properties such as protection against oxidative damage to the cell, prevention of neurodegenerative diseases, as well as many types of cancers (Custodio *et al.,* 2015; Shivapriya *et al.,* 2015). Neuroprotection refers to the strategies and relative mechanisms able to struggle down the central nervous system against neuronal damage caused by various neuropsychiatric and neurodegenerative disorders such as Alzheimer's disease, anxiety, cerebrovascular impairment, seizures, Parkinson's disease (Bachari *et al.,*

2024). Among the strategies for neuroprotection, phytochemicals may represent a valuable remedy in preventing neurodegenerative diseases. Generally, herbal products contain a variety of bioactive phytochemicals, including alkaloids, steroids, terpenoids, saponins, phenolics and flavonoids. Researchers around the globe are in search of bioactive phytochemicals from the herbs for being used as neuroprotectives in traditional medicine (Khan *et al*.,2020). Phytochemicals are naturally found and they are shown to have protective action against oxidative stress and neuroinflammation, which are major hallmarks of neurodegenerative diseases (Dash, 2021).

Tea, from the plant of *Camellia sinensis* are consumed in different parts such as green, black, or Oolong tea in worldwide. Tea has well established nutritional and medicinal properties, attractive aroma and pleasant taste due to many of the characteristic secondary metabolites present in tea leaves. The chemical composition of green tea such as phenolic compounds (30% dry weights in leave), 3–4% of alkaloids known as methylxanthine such as caffeine, theobromine and theophylline, proteins (15-20% dry weight in leave), carbohydrates (5-7% dry weight in leave) (Maslov *et al.*,2021). Phenolic compounds are major components of green tea. Most of the flavonoids are present as catechins, which possesses many beneficial effects for human health (Xia *et al.,* 2017)**.** Many researchers have reported that green tea contains catechins which possess significant antioxidant activity (Musial, *et al.* 2020). Due to the increase application of nanotechnology in various fields, comprehensive investigation into metal oxide nanoparticles has been concentrated in the past decade (Sankapal *et al.,* 2016). Among these with multifaceted benefits Zinc oxide nanoparticles are exciting inorganic materials. The nanoparticles are nontoxic and biocompatible and display excellent biomedical applications (Cai, *et al* 2016, Faisal *et al* 2021). The synthesis of green nanoparticles, especially using plant extracts is a relatively new approach in environmentally friendly chemistry that promises to be simple, non-toxic and inexpensive (Yayayuruk and Yayayuruk 2021). The rising concerns on the toxic effect of nanoparticles synthesis from green, black or Oolong tea on biological activities shifted the focus of researches to the synthesis of nanoparticles from green plants. Nanomaterials from plant extracts are reported safer in the body than chemical drugs in curing ailments (Patil and Chandrasekaran 2020). For this reason, we selected green (zinc) nanoparticles for this study. Studies have reported that zinc oxide nanoparticles from green plants has unique biological and therapeutic functions (Naveed *et al.,* 2017).

Work has already been carried out with green tea on the role of phytochemicals in neuroprotection, neurorescueing and neurodegenerative disease. Also, the roles of zinc oxide nanoparticles in various types of neuronal cell lines have been well researched. But still there is no proper treatment available for several neurodegenerative diseases. Several studies are available on zinc oxide nanoparticles for neuroprotection. Not much information is available on synthesized zinc oxide nanoparticles coated catechin. Studies have shown that polyphenols help in several neurodegenerative diseases and hence it was decided to exploit green tea rich in polyphenols to study its neuroprotective activity and employ zinc oxide nanoparticle-capped catechin from green tea to study neuroprotection.

The present study entitled '**Neuroprotective effect of synthesized zinc oxide nanoparticle-capped catechin'** was carried with the following objectives

OBJECTIVES

- To evaluate active compounds against neuroprotective target proteins using molecular docking studies
- To identify active constituents and antioxidant activity of *Camellia sinensis*
- To synthesize and characterize zinc oxide nanoparticles from *Camellia sinensis*
- To study the *in vitro* neuroprotective activity and characterize zinc oxide nanoparticle-capped catechin to neuro 2a cells

FINDINGS

The study was designed into 5 phases.

Phase I involved the evaluation of active compounds against neuroprotective target proteins using molecular docking studies.

Fifteen natural phytoconstituents such as Cyanidin, Catechin, Kaempferol Genistein, Epicatechin, Quarcetin 3-o-glucoside, Epicatechingallate, Rutin, Hesperetin, Thea flavin-3—o-gallate, Apigenin, Zinc, Herperetin, Kemopferol-3-o-rutinoside, Quercetin, Thea flavin-3'-gallate, Theaflavin, Galic acid, Caffine, Kaempferide 3-glucoside and five commercial drugs (galanthamine, rivastigmine, tacrine, memantine, and donepezil) were analysed for their interaction with neuroprotective targets through docking studies. Protein ligand interaction study has been performed through Schrodinger's software (Maestro V: 11.8 Schrodinger_suite-2019).

The crystal structure of proteins of acetylcholinesterase (AChE) (PDB ID: 5EIE 5DTI); butyrylcholinesterase (BChE) (PDB ID: 6QAA 6QAE); β-secretase (BACE) (PDB ID: 5HOX, 6UVP) and Beta amyloid (PDB ID: 4B5E 4B41). Three dimensional structures of these proteins were retrieved from the Protein Data Bank (PDB) (https://www.rcsb.org/pdb).Natural compounds and commercial drugs were docked against the drug targets acetylcholinesterase, beta-secreatase, BACE and Beta amyloid proteins. As a result, clearly showed that the Catechin and rivastigmine has been identified to have good docking with the AChE protein as determined by the docking score, number of hydrogen bonds formed and the number of good contacts. Compared to the other commecially available drugs and natural compounds, rivastigmine, and catechin respectively were found to be most effective in binding to the AChE active site because of their significantly low binding affinities as a result of high docking score values. Based on the above results, catechin rich plant source (*Camellia sinensis*) was selected for further studies

In **Phase II** characterization of *Camellia sinensis* and identification of its bioactive compound

The *Camellia sinensis* (L.) Kuntze leaves were collected from Aruvankadu in the Distirct of Niligris and the taxonomic identification of the plant was confirmed by Botanical Survey of India, Coimbatore (Aunthentification No: BSI/SRC/5/23/2019/Tech./185). *Camellia sinensis* leaves were done the preliminary phytochemical analysis, antioxidant and free radical scavenging activities, identification of bioactive compound by spectral and chromatographical methods. The results revealed that the hydroethanolic extract of *Camellia sinensis* had more phytochemicals compared to the hexane, ethyl acetate, methanol & ethanol extracts. *Camellia sinensis* leaves had higher amounts of enzyme and non-enzymic antioxidant activities. Hence, it is evident that the hydroethanolic extract of *Camellia sinensis* can be separated and identified by column and thin layer chromatographic techniques and that the 3rd chromatographic fraction of the sample contains catechin the active constituent in *Camellia sinensis*. Since the active component was identified in the 3rd column chromatographic fraction of the hydroethanolic extract of *Camellia sinensis*, this fraction was further subjected to HPTLC, UV, FT-IR, GC-MS, NMR and LC-MS to identify the component. The results showed that the active component was isolated from the hydroethanolic extract of *Camellia sinensis*, chromatographic and spectral analysis of the selected fraction confirmed the bioactive component to be catechin. From the overall results of Phase II it can be concluded that *Camellia sinensis* leaves contains a good

amount of antioxidants and radical scavenging activites. The main active component was identified to be Catechin.

Phase III included characterization of synthesized zinc oxide nanoparticles from *Camellia sinensis*

The zinc oxide nanoparticles synthesized by *Camellia sinensis* and characterized by the UV, FTIR, SEM, XRD & zeta potential. In additionally antioxidant activity was carried out using zinc oxide nanoparticle. Inhibition of Acetylcholinesterase (AChE) inhibitory activity of synthesized zinc oxide nanoparticles has been demonstrated. Thin layer chromatography (TLC) with bioassay detection for AChE inhibition assay has been demonstrated. The results on the UV spectra of synthesized zinc oxide nanoparticles showed characteristic peak at 280 nm. During the synthesis of zinc oxide nanoparticles, with increase in the time of exposure the colour changed from brown to white. The XRD peaks were broad nature which could be attributed to the nano crystalline nature of the synthesized zinc oxide nanoparticles. The SEM images of synthesized zinc oxide nanoparticles particles were flower shaped and found to be in aggregates at nanoscale. The FT-IR result also confirmed the formation of synthesized zinc oxide nanoparticles by showing prominent absorption peaks. The Zeta potential spectrum showed the stability of synthesized zinc oxide nanoparticles and the synthesized zinc oxide nanoparticles were found to have the IC50 value of the synthesized zinc oxide nanoparticles was found to be 70.37 %. Concentration of 1.25 µg/ml in the synthesized zinc oxide nanoparticles showed the most potent effect in AChE inhibition activity. From the overall finding of Phase III, it can be concluded that the remarkable potential of synthesized zinc oxide nanoparticles from *Camellia sinensis* makes it a valuable source of antioxidants with acetylcholinesterase inhibitory activity

Is **Phase IV** involved synthesized zinc oxide nanoparticle-capped Catechin their characterization and *in vitro* Neuroprotective activity

The synthesized zinc oxide nanoparticles are coated isolated bioactive compound catechin were synthesized through nanoprecipitation method in which the stability and characteristics of synthesized zinc oxide nanoparticle-capped catechin including topography, morphology, composition and crystalline structure, drug loading capacity and entrapment efficacy, *in vitro* release analysis and acetylcholinestrease activities has been optimized. UV and visible range spectra of synthesized zinc oxide nanoparticle-capped catechin showed a characteristic peak 318 nm indicating that synthesis of synthesized zinc oxide nanoparticle-

capped catechin increased with increasing time of exposure, confirming that the immediate colour change was due to the synthesis of nanoparticles. The broad nature of the XRD peaks could be attributed to the nano crystalline nature of the synthesized zinc oxide nanoparticle-capped catechin SEM results proved that synthesized zinc oxide nanoparticle-capped catechin particles were completed coated in the zinc oxide nanoparticles with the distribution of similar shape and size of the solution and found to be in aggregates at nanoscale. The FT-IR results also confirmed the formation of synthesized zinc oxide nanoparticle-capped catechin by showing prominent absorption peaks. The results revealed that the DPPH activity was assessed. *In vitro* release study and acetylcholinestrease inhibitory activity proved maximum inhibitory activity of acetylcholinestrease. The therapeutic activity of synthesized zinc oxide nanoparticle-capped catechin based upon their moderate acetylcholinestrease inhibitory activity in combination with minimal off-target effects. From the overall finding of Phase IV, it can be concluded that there is a remarkable potential of synthesized zinc oxide nanoparticle-capped catechin making it a valuable source of antioxidants with acetylcholinesterase inhibitory activity and also had good encapsulation efficiency and *in vitro* release.

During **Phase V** the *in vitro* neuroprotective activity of synthesized zinc oxide nanoparticle-capped catechin to neuro2a cells

The results *in vitro* neuroprotective effect of synthesized zinc oxide nanoparticle-capped catechin was evaluated against neuro2a cells, by MTT cell proliferation assay, AChE inhibitory assay and also apoptotic effect of neuro2a cells by Flow Cytometry. The results of MTT assay showed that synthesized zinc oxide nanoparticle-capped catechin mediated a concentration depended increases in toxicity towards neuro 2 a cells with the concentration of 20 g/mL of synthesized zinc oxide nanoparticle-capped catechin there were no significant changes in the percentage of cell viability. The *in vitro* cell line culture study reported that the synthesized zinc oxide nanoparticle-capped catechin have neuroprotective activity of neuro 2 a cells line sustained manner. The study of the *in vitro* cytotoxic effect of synthesized zinc oxide nanoparticle-capped catechin on neuro-2a cells showed less toxicity. Synthesized zinc oxide nanoparticle-capped catechin increased the production of intracellular ROS in a dose-dependent manner in neuro-2a cells. The most potent inhibitor for acetylcholinestrease was the zinc oxide nanoparticle-capped catechin because it had the lowest inhibitory activity. From the overall outcome of Phase V it can be concluded that exposure of neuro 2a cell lines to synthesized zinc

oxide nanoparticle-capped catechin, reduced the neurotoxic effects including changes of cell morphology, cell viability, ROS production on neuro2a cells.

It can thus be concluded that the synthesized zinc oxide nanoparticle-capped catechin can be proposed as a novel therapeutic strategy for neuroprotective activity

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